

Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QToF-MS/MS)

Overview

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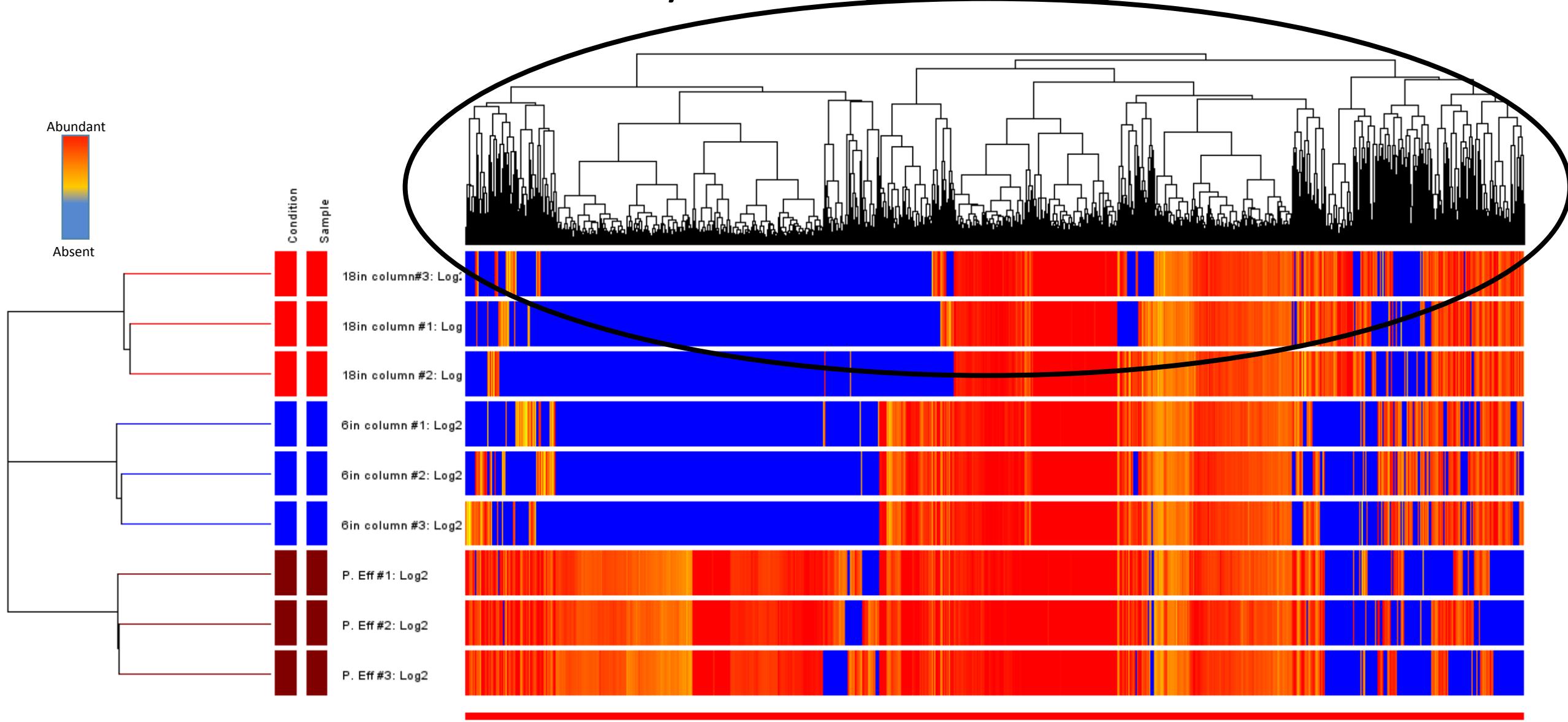
LC-QToF-MS/MS

Applications

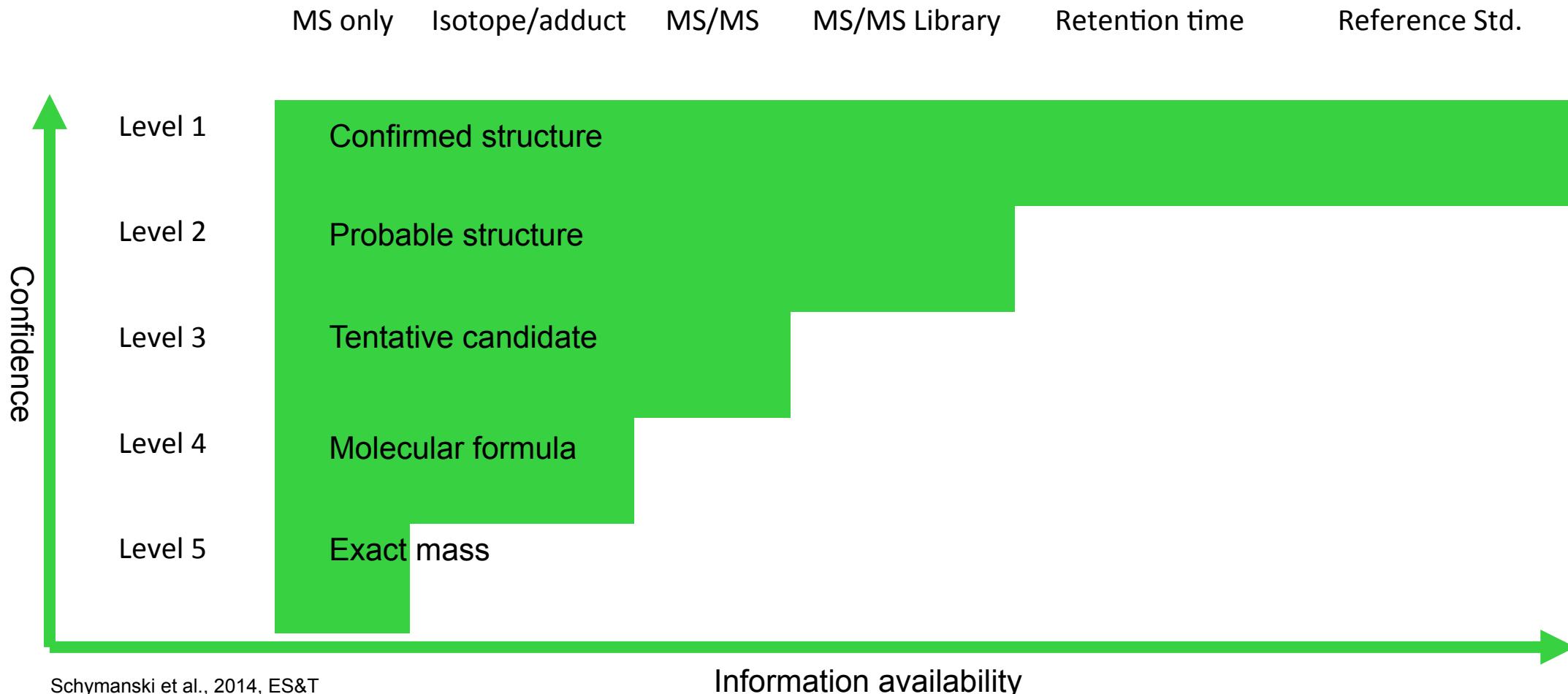
- Screening
 - There are thousands of compounds being used - environmental information on only a limited number of them.
- Identification of related compounds (metabolites and variants)
 - We know that some compounds may occur in environment. However, metabolites/transformation products/variants may be important.
- Comparisons
 - Systematic



QToF Data – Identify Multiple Compounds

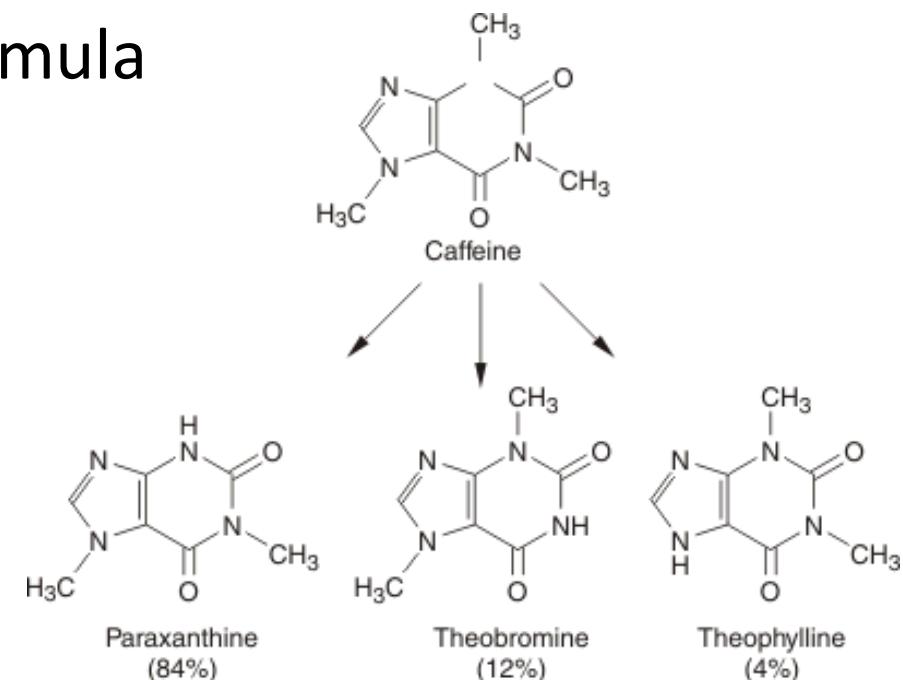


Confidence of Identification – Screening Unknowns



QToF Data

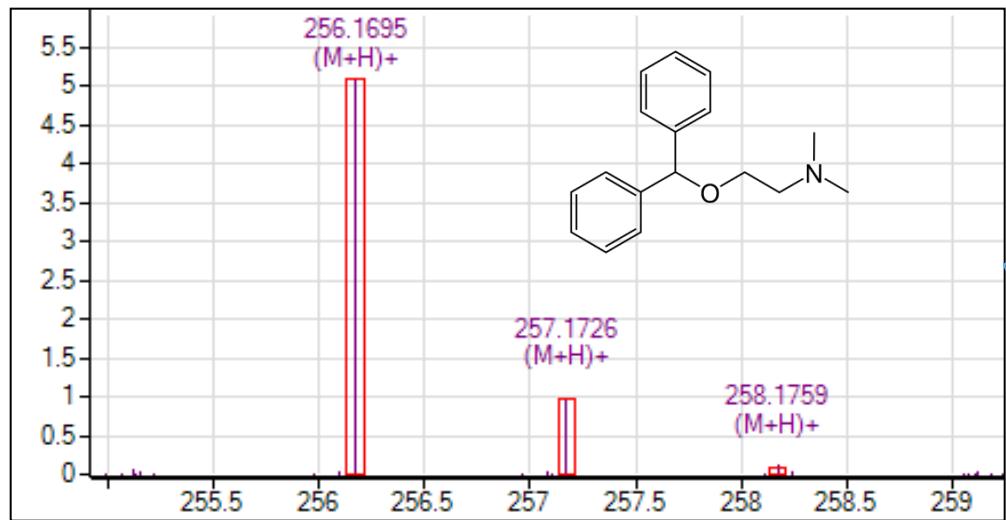
- Accurate Mass (MS QToF)
 - Information on the mass of a compound (and isotope patterns) allows the determination of a molecular formula
 - Molecular Weight = 194.0803 → C₈H₁₀N₄O₂ → Caffeine (?)
 - May be a suite of compounds with same formula
 - Molecular Weight = 180.0647 → C₇H₈N₄O₂ → Theobromine/Paraxanthine/Theophylline
 - Database with > 70,000 compounds



QToF Data

MS/MS library

Diphenhydramine

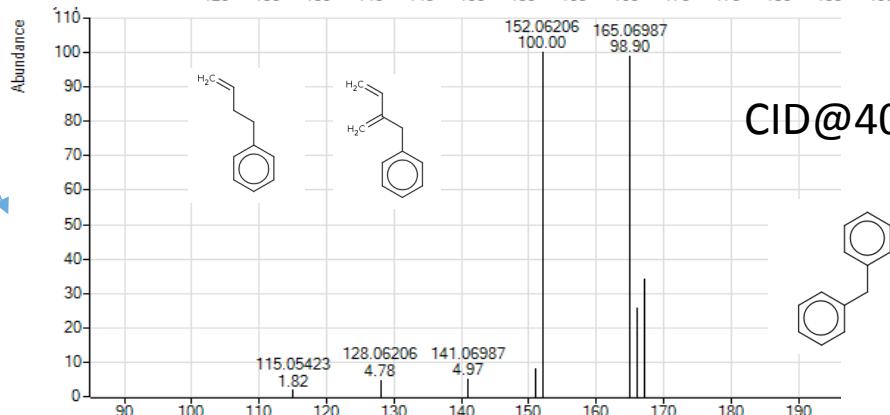
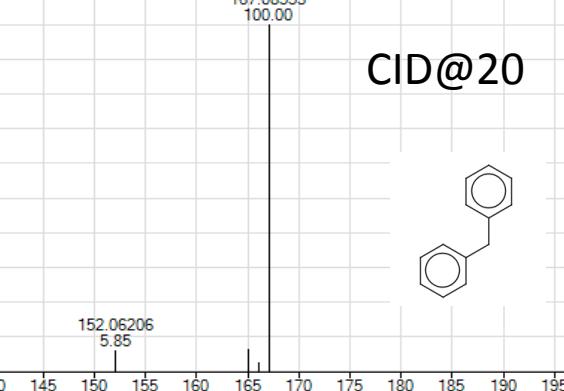
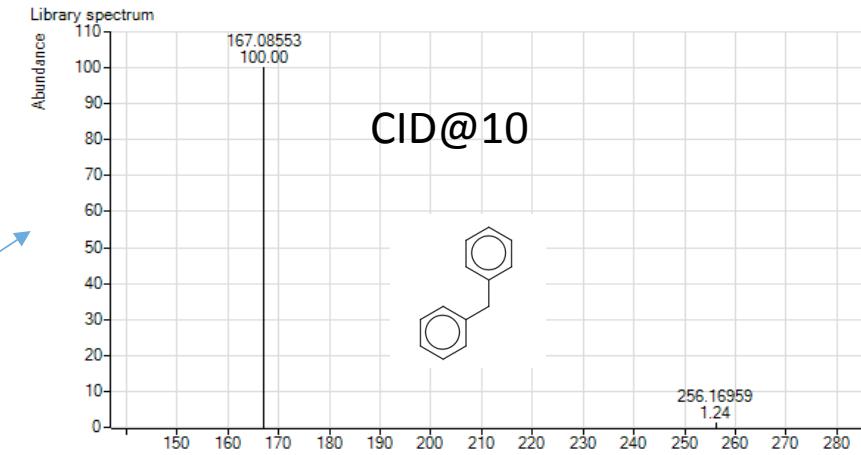


Accurate Mass

5 Compounds with

Same Formula

$C_{17}H_{21}NO$



Biotoxins in Puget Sound

Preliminary Investigation

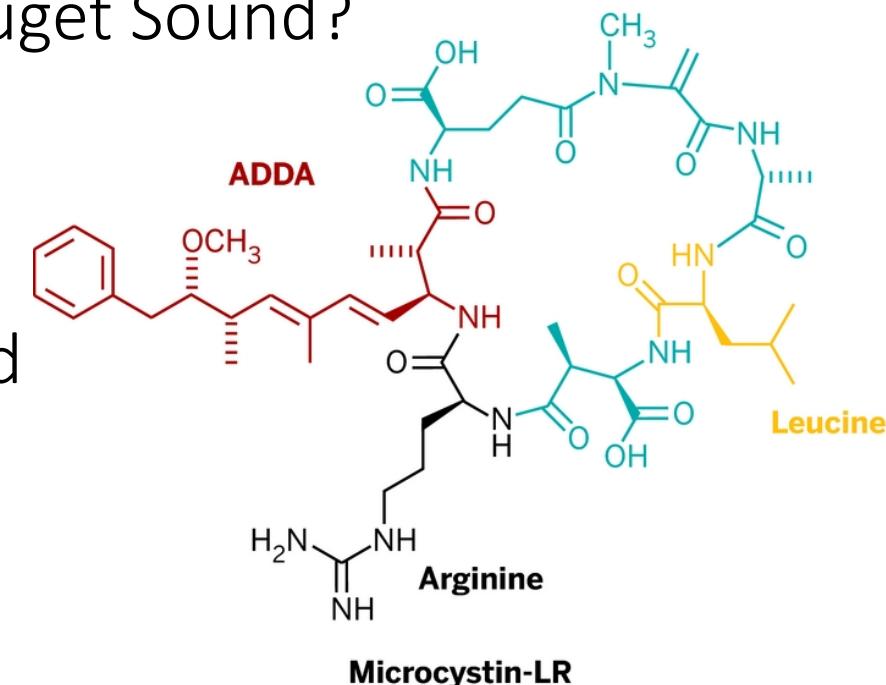
- Are Azaspiracids occurring in Puget Sound waters?
 - A genetic signal has been detected but no compounds
- What other marine biotoxins are in Puget Sound?
- Is there evidence of freshwater biotoxins in Puget Sound?

Process

NOAA deployed passive samplers in Puget Sound

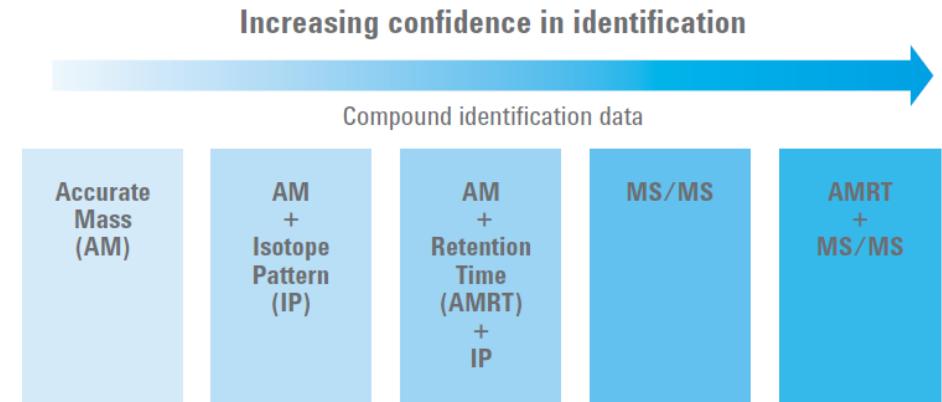
US FDA Maryland extracted in methanol

Supplied UWT sample extract



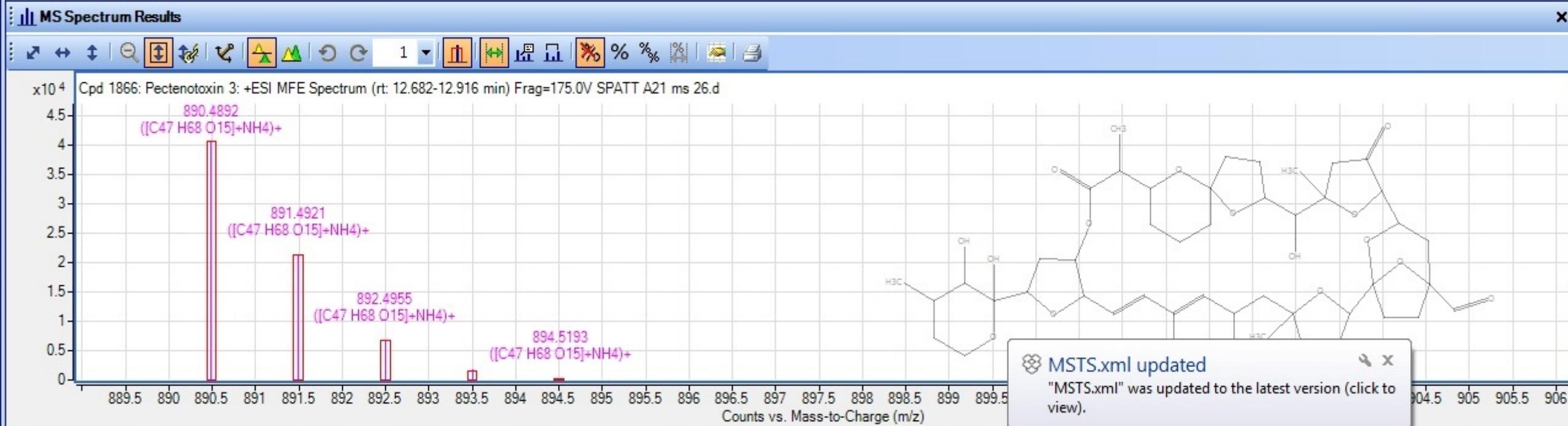
Biotoxins – Non targeted screening

- Gather accurate mass data on largest number of features as possible to identify potential candidates of interest.
 - MS-only acquisition and processing identified 6000 compounds.
- Evaluate MS-only data
 - Retention time data can eliminate nonsensical identifications.
 - Develop focused database with compounds of interest. e.g., biotoxins database with ~ 70 compounds
- Analyze samples with semi-targeted MS/MS
- Screen MS/MS data for potential identifiers



Automatically Show Columns | Cpd | File | ID Source | ID Techniques Applied | Name | m/z | Formula | Flags (Tgt) | Saturated | Start | RT | End |

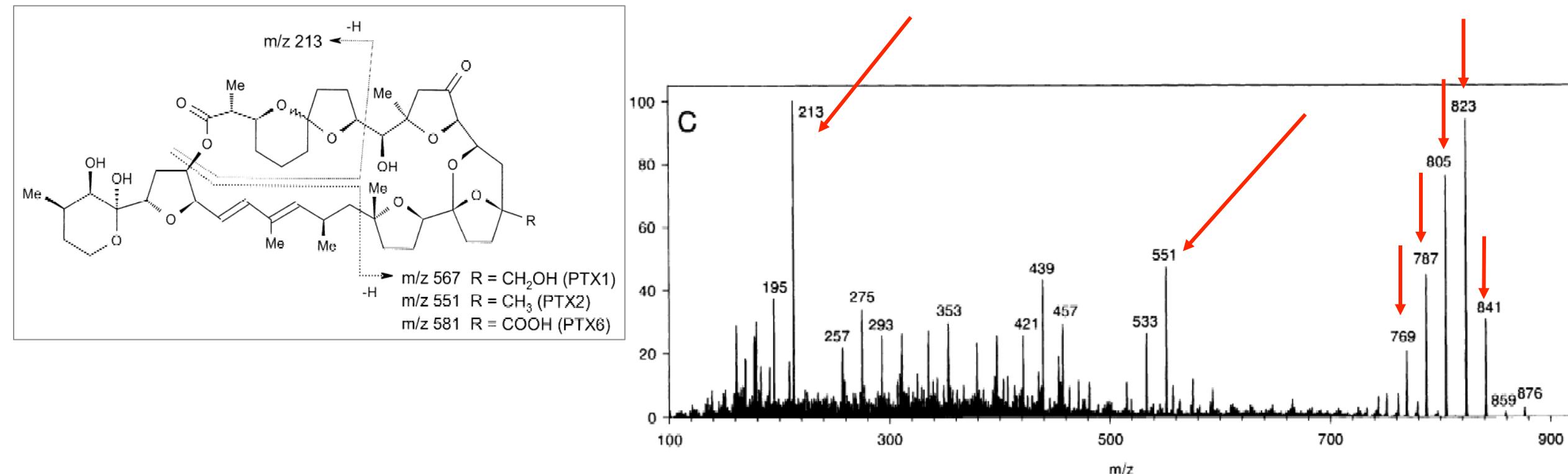
	Cpd	File	ID Source	ID Techniques Applied	Name	m/z	Formula	Flags (Tgt)	Saturated	Start	RT	End
<input checked="" type="checkbox"/>	1866	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 3	890.4892	C47 H68 O15			12.682	12.796	12.916
<input type="checkbox"/>	1843	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 2	876.5108	C47 H70 O14			12.549	12.641	12.716
<input type="checkbox"/>	1845	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 2 secoacid	881.4661	C47 H72 O15			12.56	12.643	12.716
<input type="checkbox"/>	2053	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 2 secoacid	876.5097	C47 H72 O15			13.7	13.77	13.816
<input type="checkbox"/>	1683	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 1	892.505	C47 H70 O15			11.42	11.495	11.516
<input type="checkbox"/>	1958	SPATT A21 ms 26.d	DBSearch	DBSearch	Dinophysistoxin 1	841.4705	C45 H70 O13			13.211	13.277	13.316
<input type="checkbox"/>	1878	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 2 secoacid	876.5096	C47 H72 O15			12.76	12.863	12.916
<input type="checkbox"/>	2078	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 2 secoacid	876.5097	C47 H72 O15			13.856	13.921	13.946
<input checked="" type="checkbox"/>	1	SPATT A21 ms 25.d	FBF	FBF		890.4889	C47 H68 O15			12.68	12.796	12.916
<input checked="" type="checkbox"/>	1	SPATT A21 ms 27.d	FBF	FBF		890.4889	C47 H68 O15			12.692	12.798	12.916
<input type="checkbox"/>	1827	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 2 secoacid	894.5202	C47 H72 O15			12.36	12.492	12.516
<input type="checkbox"/>	1685	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 1	897.4602	C47 H70 O15			11.431	11.499	11.516
<input type="checkbox"/>	2002	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 3	890.4889	C47 H68 O15			13.383	13.452	13.476
<input type="checkbox"/>	1675	SPATT A21 ms 26.d	DBSearch	DBSearch	Pectenotoxin 1	892.5055	C47 H70 O15			11.325	11.404	11.426

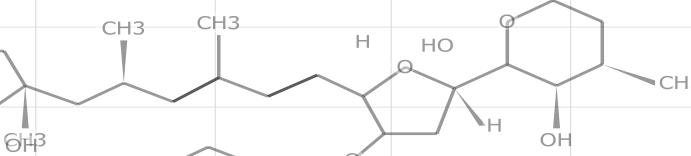
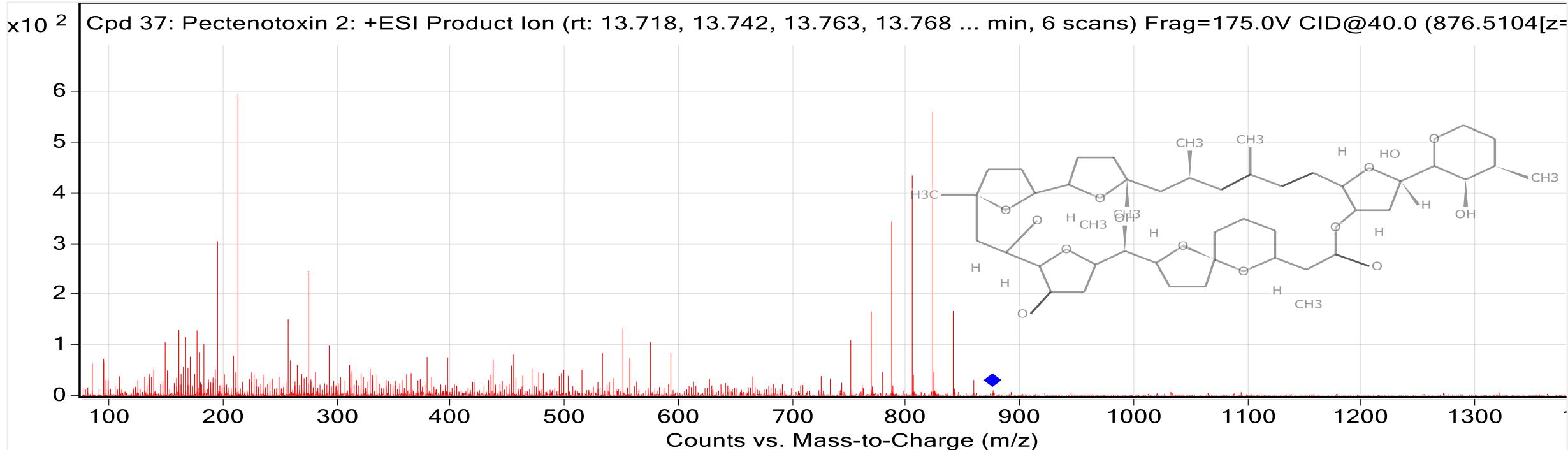


Strategies for Strengthening Confidence of Identification Related Compounds without Standards

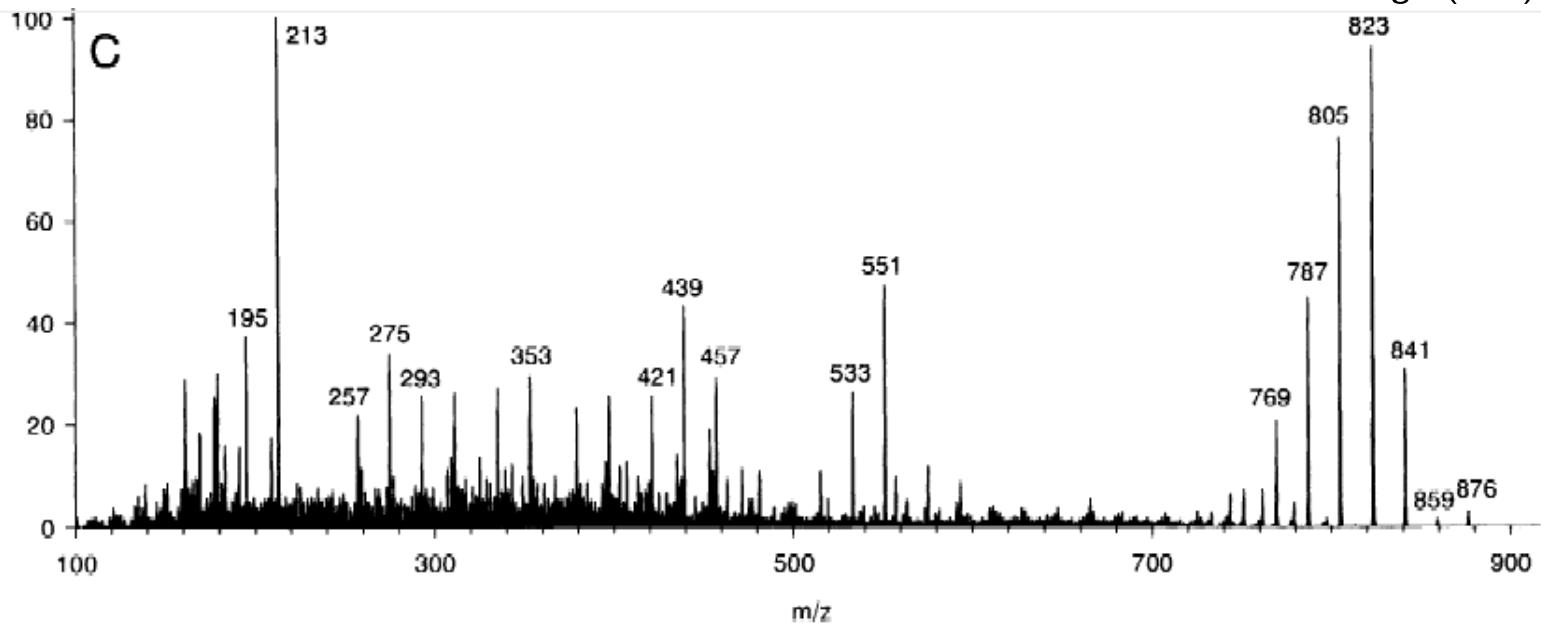
Fragment Screening and Fragment Patterns

- Pectenotoxins appear to have a common fragment ($m/z = 213$)
- Pectenotoxins appear to have a related fragment (varies by oxidation of methyl group)
- Pectenotoxins undergo series of water losses





Also identified PTX 3 based on initial mass/isotope patterns in addition to MS/MS fragmentation.



Biotoxins – Summary

- No evidence of known Azaspiracids or Microcystins in selected samples
- Confirmed presence of DTX 1 (MS and MS/MS)
- Identified biotoxins that had not been previously noted in Puget Sound waters

Next Steps

- Evaluate occurrence patterns
 - Biotoxins that are generally not part of analytical focus
 - variants
- Evaluate degradation pathways

NOTES

Biotoxins – Illustrative Example

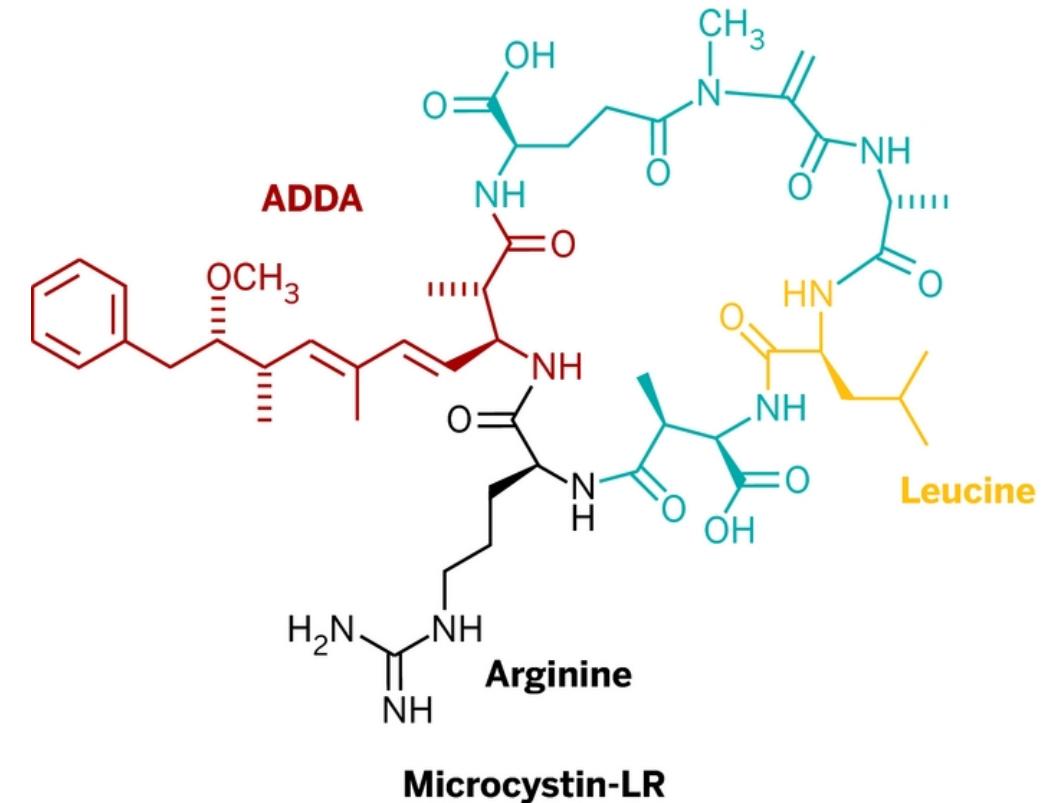
Marine and freshwater organism can produce a wide array of biotoxins

Freshwater

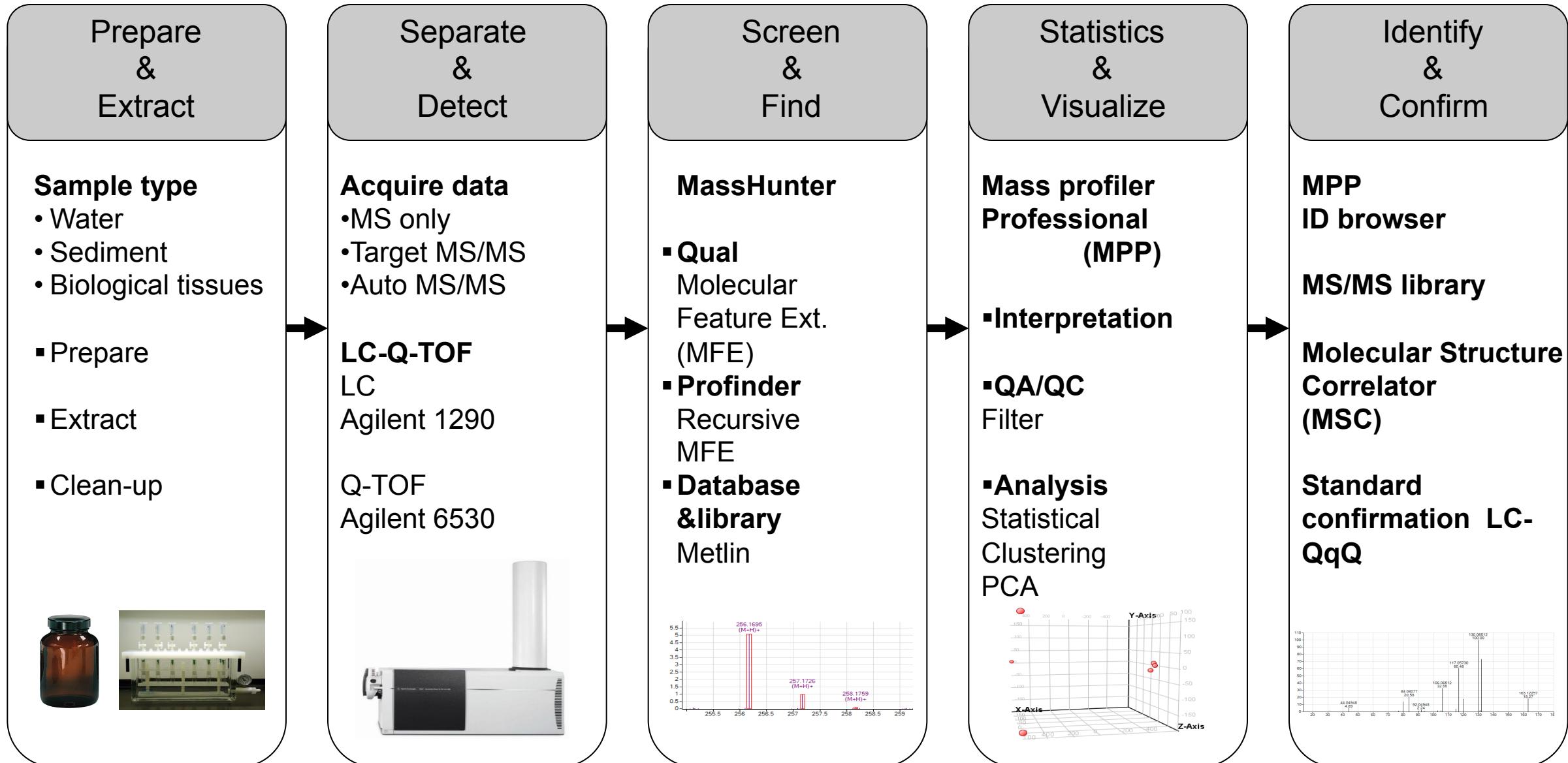
- Microcystin – 80+ variants
- Nodularins – 9+ variants
- Cylindrospermopsim – 2 variants

Marine Water

- Okadiac acid + dinophysistoxins
- Domoic acid
- Saxitoxins
- Azaspiracids – 15+ variants

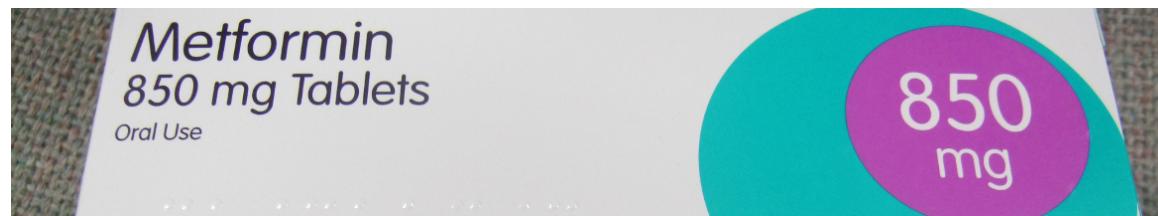


Non-target Analysis using Q-TOF LC/MS at CUW (summarized from preliminary investigations...)



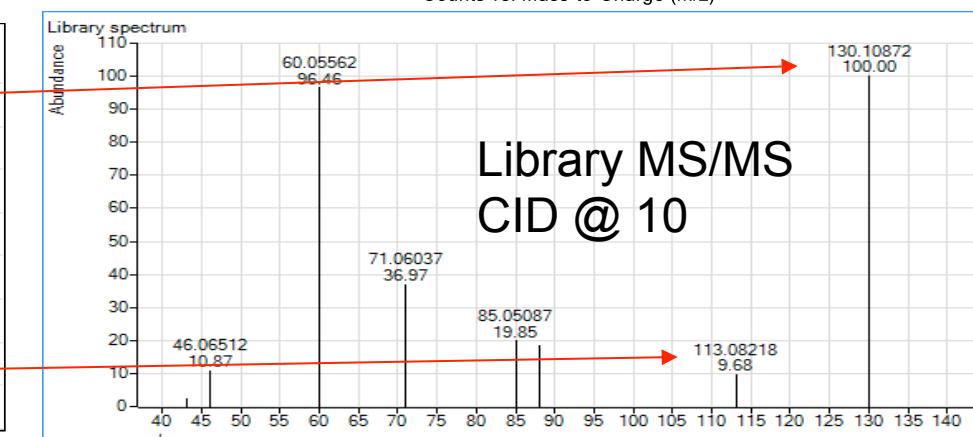
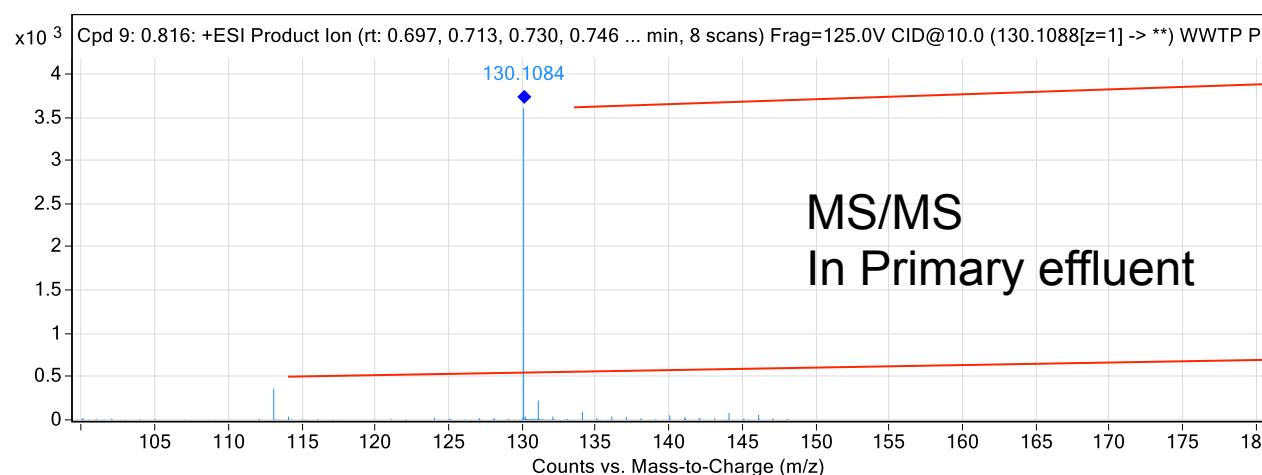
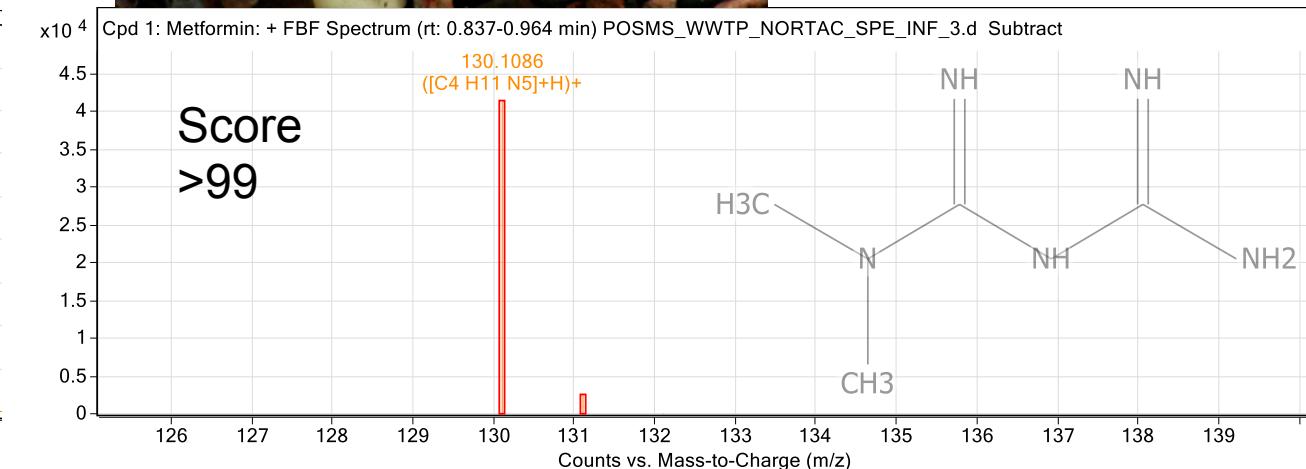
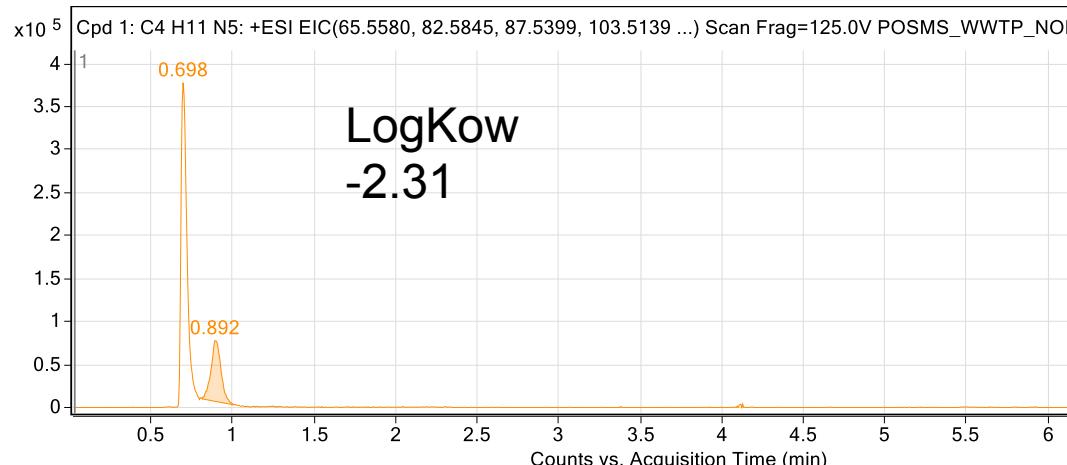
Preliminary data analysis, example 1

Metformin in the wastewater (confidence: level 2)



Dr. Rebecca Klaper
UWiscosin-Milwaukee

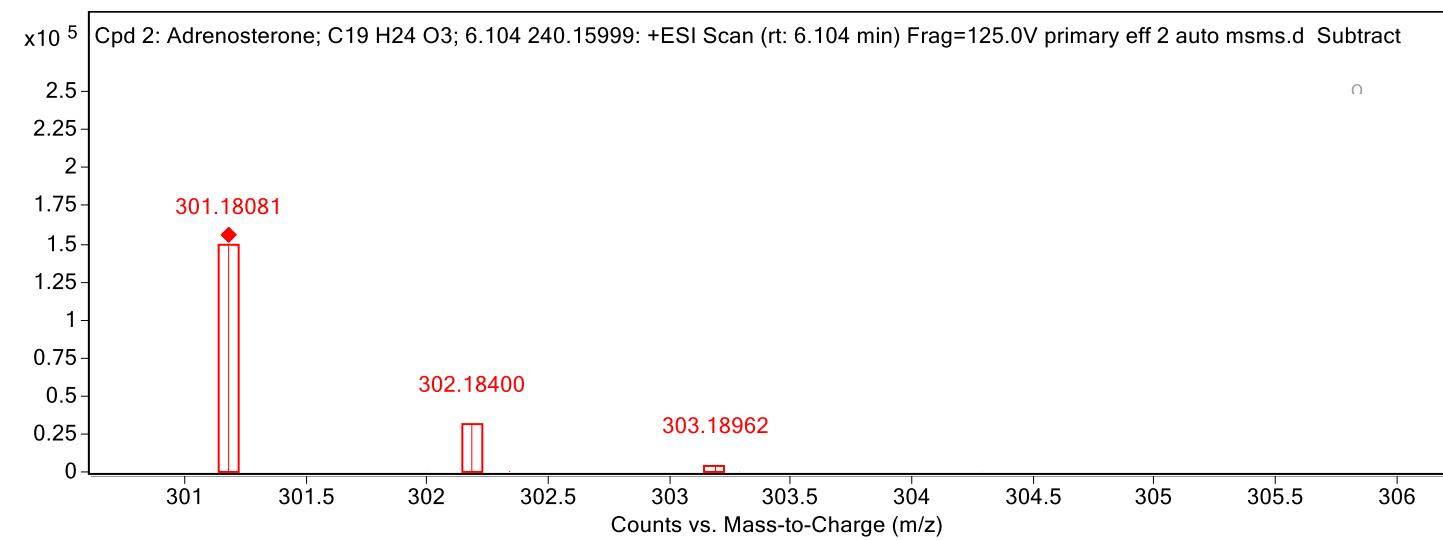
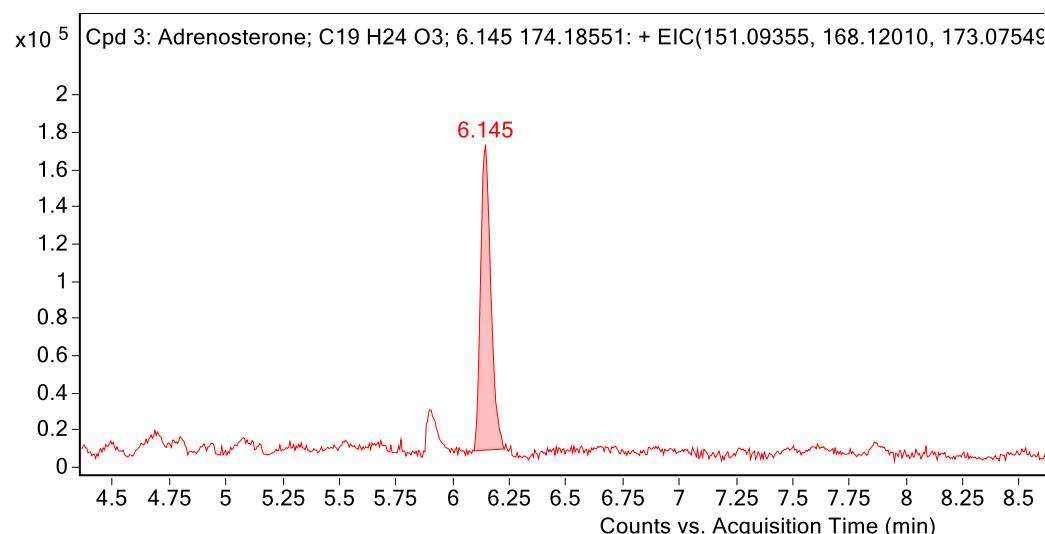
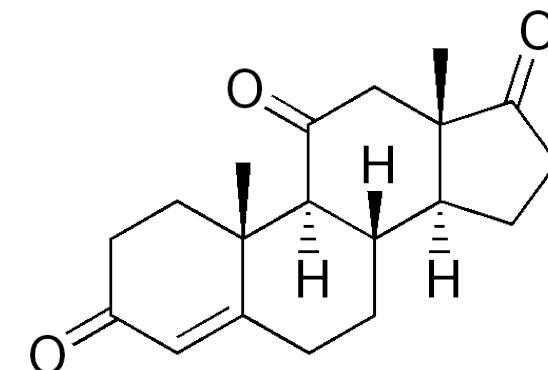
Exposure to widespread diabetes drug feminizes male fish



University of Iowa list of GCs

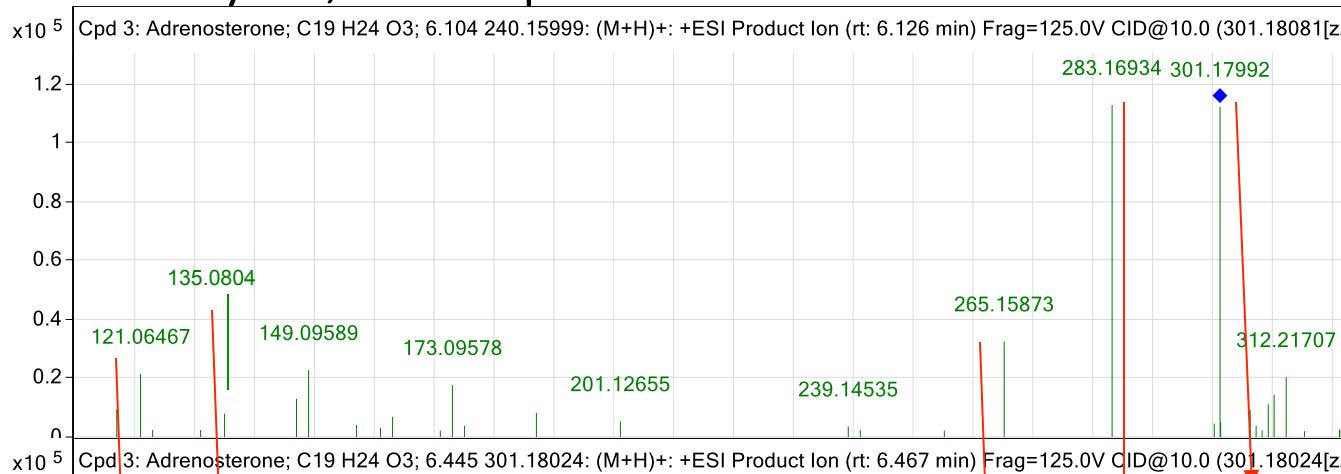
Compound	Formula	Mass	<i>m/z</i> (+H)	Ref Std
Adrenosterone	C ₁₉ H ₂₄ O ₃	300.1725	301.1798	Yes
Δ1-Adrenosterone	C ₁₉ H ₂₂ O ₃	298.1569	299.1642	Yes
Chloro-Δ1-Adrenosterone	C ₁₉ H ₂₁ ClO ₃	332.1179	333.1252	
Cortisol	C ₂₁ H ₃₀ O ₅	394.1547	395.1620	Yes
Dichloro-cortisol	C ₂₁ H ₂₈ Cl ₂ O ₅	430.1314	431.1387	
Cortisone	C ₂₁ H ₂₈ O ₅	360.1937	361.2010	Yes
Dexamethasone	C ₂₂ H ₂₉ FO ₅	392.1999	393.2072	Yes
11-oxo-dexamethasone	C ₂₂ H ₂₇ FO ₅	390.1843	391.1915	
17-oxo-dexamethasone	C ₂₀ H ₂₅ FO ₃	332.1788	333.1860	
11β-hydroxyboldione	C ₁₉ H ₂₄ O ₃	300.1725	301.1798	
Prednisolone	C ₂₁ H ₂₈ O ₅	360.1937	361.2010	Yes
Chloro-prednisolone	C ₂₁ H ₂₇ ClO ₅	394.1547	395.1620	
Prednisone	C ₂₁ H ₂₆ O ₅	358.1780	359.1853	Yes
9-Chloro-prednisone	C ₂₁ H ₂₅ ClO ₅	392.1391	393.1463	

Adrenosterone

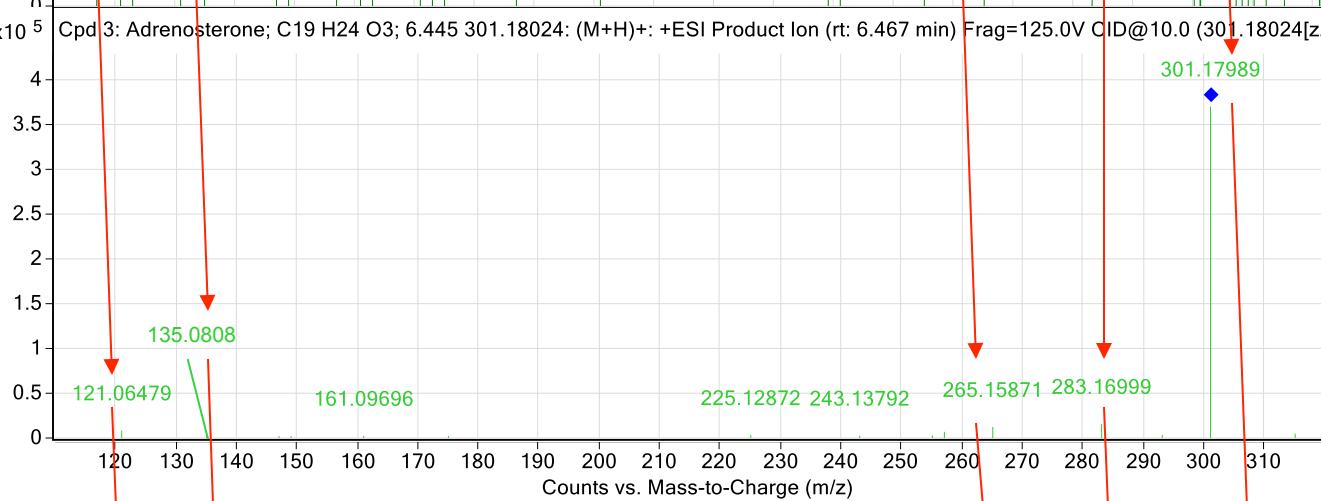


Preliminary data analysis, example 2

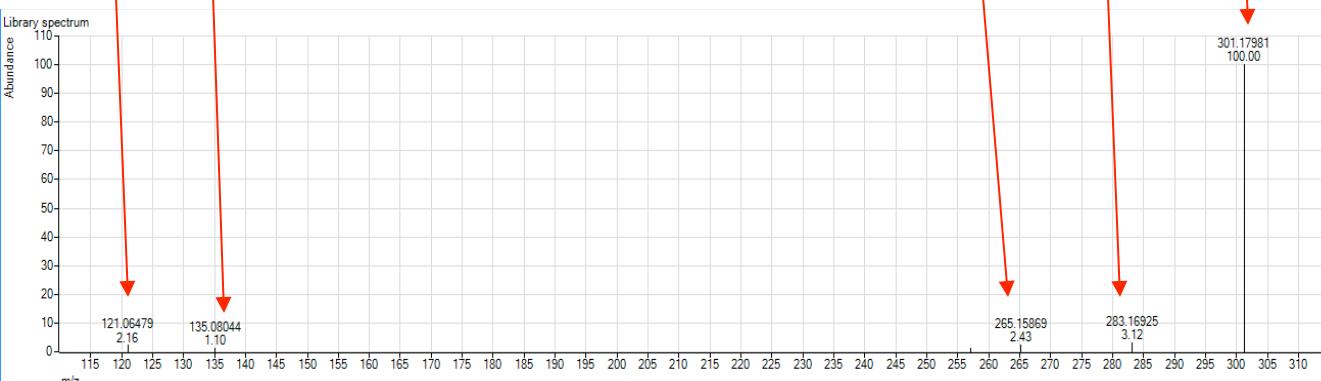
Primary effluent



Reference standard



Library



MS/MS spectra of Adrenosterone in the primary effluent, reference standard, and library, Confidence: Level 1

